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APPLICATION OF SILVER NANOPARTICLES IN VIRAL INHIBITION: A NEW HOPE FOR ANTIVIRALS

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In modern era, viral infections constitute one of the main health problems. Chemically synthesized antiviral drugs have adverse side effects which are associated with other health complications. The emergence of new infectious diseases and increase in frequency of drug resistant viruses demand the most effective and novel therapeutic agents, one of which are nanoparticles. Nanoparticles excel as therapeutic agents due to their unique physiochemical properties and a universally applicable physical mode of action. Their large surface area to volume ratio offers them an edge as an antiviral agent. The review focuses on the mechanism of action of silver nanoparticles and their novel therapeutic applications against some viruses bearing potentially fatal consequences.

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1. Introduction

Viruses infect all cellular life forms; eukaryotes (vertebrate animals, invertebrate animals, plants, fungi) and prokaryotes (bacteria and archaea) (1). The infection of virus in prokaryotes is prevalent in the form of bacteriophages. Viruses form the most substantial causes of disease and death in the world. The presence of viruses in the host organisms shows some manifestations of disease. Many healthy organisms are hosts of non-pathogenic virus infections; a few are active, while others are found dormant. Moreover, many organisms' genomes contain residual primordial viral genomes that integrated into their host genomes long ago. Viruses are also found in all forms of life i.e. air, water and most importantly in the soil. Owing to massive vaccination drives, some of the diseases which inflicted death, misery, disfigurement and permanent disability have been eradicated, such as smallpox in 1979 (2), paralytic poliomyelitis (from many parts of the world) (3). Some diseases which have been eradicated with the help of the vaccines are listed in Table 1.

However, for today's most pressing viral pathogens, there is still no vaccine available. A large number of viruses are prevalent today such as *HIV, Rhinovirus, Hepatitis C, influenza*, etc. A horde of problems are associated with *herpes viruses;* from *shingles, genital herpes*, chickenpox, infectious mononucleosis, up to *herpes keratitis*, neonatal disseminated infections and viral encephalitis. Viruses are known to cause extensive suffering and persistent infections that may lead to cancer or acquired immunodeficiencies, such as hepatitis viruses (mainly HBV and HCV) or *Human immunodeficiency virus* (HIV). An increasing number of attempts are being made to develop vaccines for such deadly diseases, without complete success. Virology is a very fast emerging field so the discovery of new antiviral compounds forms a promising research area (4). The present attempt is to emphasize the antiviral mechanism of silver nanoparticles and their incorporation into novel therapeutic approaches against deadly viruses prevalent today.

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Table 1: List of diseases and their eradication (1-3, 5, 6)

Disease	**Virus Name**	**Family**	**Eradication**	**Vaccine**
Small pox	*Variola virus*	*Poxviridae*	Completely in 1980	Smallpox Vaccine
Poliomyelitis	*Human enterovirus C*	*Picornaviridae*	Eradicated In some countries	OPV IPV
Cowpox	*Cowpox virus*	*Poxviridae*	Completely in 1980	Smallpox Vaccine
Rinderpest	*Rinderpest Virus*	*Paramyxoviridae*	Completely in 2011	RPVINS-GFP RPVSIG-GFP
Hookworm	*N. americanus A. duodenale*	*Anyclostomatidae*	Not eradicated	Hookworm Vaccine

2. Nature of viruses

The genome of eukaryotic cells is mostly composed of double stranded DNA. There are four possibilities for a virus genome (A) Double-Stranded DNA, (B) Single-Stranded DNA, (C) Double-Stranded RNA, and (D) Single-Stranded RNA genome. The genome of virus is enclosed in a protein coat known as a capsid. In most cases, the genome, capsid, and other components constitute the virions. The function of the virion is to protect the genome and to deliver it into a cell in which it can replicate. Generally, viral genomes are much smaller than cell genome. Viruses encode all their requirements in a small genome. Viruses achieve this in a number of ways (1).

1. Host cell proteins: The genome of large viruses duplicates some of the functions of the host cell, but the small viruses rely very heavily on functions of the host cell. On the other hand, one of the factors that an RNA virus must encode is an RNA polymerase, because cells do not produce enzymes that can replicate viral RNA. A considerable percentage of the genome of an RNA virus is taken up with the gene for an RNA polymerase (1).
2. Viruses code efficiently: There may be overlapping genes and genes encoded within genes. The small genome of hepatitis B virus is a good example of efficient coding (1).
3. Many virus proteins are multifunctional**:** A single viral protein may have several enzyme activities (1).

Viruses come into view because of changes in the host, in the environment, or by the vector, and sometimes new virulent species can arise in humans from existing human viruses or from animal viruses. Numerous viral diseases prevalent since the last few decades have now become embedded in human populations worldwide. The major examples are: SARS corona virus (7), monkey pox virus (8), Chikungunya virus (9), and pandemic influenza viruses most recently of avian or swine origin (4, 9, 10).

Many improvements have been made in antiviral therapy, but due to ineffectiveness new antiviral agents are urgently needed to fight these deadly viruses. Owing to the boost in technology, frenzy is ongoing for the discovery and characterization of molecules required for viral replication and the development of antiviral agents to inhibit them. Most viruses are indeed provided with an extraordinary genetic adaptability, which has enabled them to escape antiviral inhibition and in certain cases to regain advantage over the host by mutagenesis that creates new viral strains with acquired resistance to most of the antiviral compounds available (4).

2.1 Interaction of virus and host cell:

Virus infection is characterized by composite interactions between the virus and the host cellular system. All viruses depend upon a host cell for their protein synthesis. As shown in figure 1, first the binding of a virus to the host cell takes place and then the genome of virus enters into the cytoplasm. This genome is liberated from the capsid and transcribed in the cytoplasm or in the

nucleus. Viral mRNA and proteins are synthesized. Next, the replication of viral genome takes place and assembly of progeny virions takes place with the help of the viral structural proteins which are released from the cell. Each phase represents possible inhibitory targets. Early step targeting is very fascinating venture for therapeutic invention because the inhibitory action site is extracellular and consequently relatively accessible; this could be paired by a concomitant action of the same drug on multiple targets to obtain a more effective therapeutic compound. Viral inhibition can be carried out in every step of viral replication and expression. Targeting the early step of viral infection is promising approach because the site of the action of inhibitor is extracellular and accessible, but it is very difficult to target early steps of virus attachment (11).

3. Nanotechnology

Nanotechnology deals with structures ranging from approximately 1 to 100 nm in at least one dimension. The field of nanotechnology is one of the most active areas of research in contemporary materials science. Nanoparticles have completely new or improved properties which are based on specific characteristics like size, shape, crystalline structure and morphology. This field is emerging with new discoveries every day, making an impact on all spheres of human life. The potential of nanoparticles and nonmaterial are emerging rapidly(12, 13). Silver in nano-crystalline form has found marvelous applications in the field of high sensitivity bio molecular detection, disease and chemical diagnostics, antimicrobial and therapeutic compounds, catalysis and micro-electronics. Nanoparticles have also been used in consumer products, as well as electronics, sensors, munitions, and propulsion technologies (14).

3.1 Silver as an antimicrobial agent

Silver has been in use since time immemorial. However the use of silver for medicine or as a local antibacterial agent was not recognized until the nineteenth century. Since then the antimicrobial property of silver has been investigated and employed more extensively than any other inorganic antibacterial agent. As early as 1000 B.C. silver was used to make water potable. Solid form of Silver nitrate was used for the treatment of various diseases, diseases of salivary glands and bone and perennial abscesses. In the 19^{th} century granulation tissues were removed using silver nitrate to allow epithelization and promote crust formation on the wound surface. A different concentration of silver nitrate was used for the treatment of fresh burns. In the 1940s, after penicillin was introduced, the use of silver for the treatment of bacterial infections minimized. Silver again came into picture in the 1960s when Moyer introduced the use of 0.5% silver nitrate for the treatment of burns. He proposed the antibacterial property of silver against *Staphylococcus spp., Pseudomonas spp.* and *Escherichia coli*. Silver nitrate was merged with sulfonamide to form silver sulfadiazine cream, which used as a broad-spectrum antibacterial compound and was used for the treatment of burn victims. Silver sulfadiazine is effective against bacteria like *E. coli, S. aureus, Klebsiella spp.,* and *Pseudomonas spp.* Due to the increasing bacterial resistance to classic antibiotics, the investigations on the antibacterial activity of silver nanoparticles have amplified (14, 15).

3.2 Metallic silver

It is static in its metallic state but reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is extremely reactive, as it binds to tissue proteins and brings structural changes in the cell wall of bacteria and nuclear membrane leading to cell distortion and cell death. Silver binds to microbial genome (DNA or RNA) by denaturing and inhibits its replication (14). Silver vessel is also used to make water potable which becomes sterile. As the concentration of Ag^+ ion is very low, this has been called oligodynamic action (16).

3.3 Mechanism of action

The action of silver on the microbes is exactly not known but the possible mechanism of action of metallic silver, silver ions and silver nanoparticles has been suggested according to the morphological and structural changes found in the bacterial cells.

3.4 Mechanism of silver in the cell

The mechanism of action of silver is associated with its interaction with thiol group which is found as a functional group in the respiratory enzymes of bacterial cells. Silver gets attached to the cell wall and membrane and then it inhibits the respiration process (17). In case of *E. coli*, silver acts by inhibiting the uptake of phosphate and releasing carbohydrates like mannitol, succinate, and amino acids like proline and glutamine and phosphate from *E. coli* cells (18).

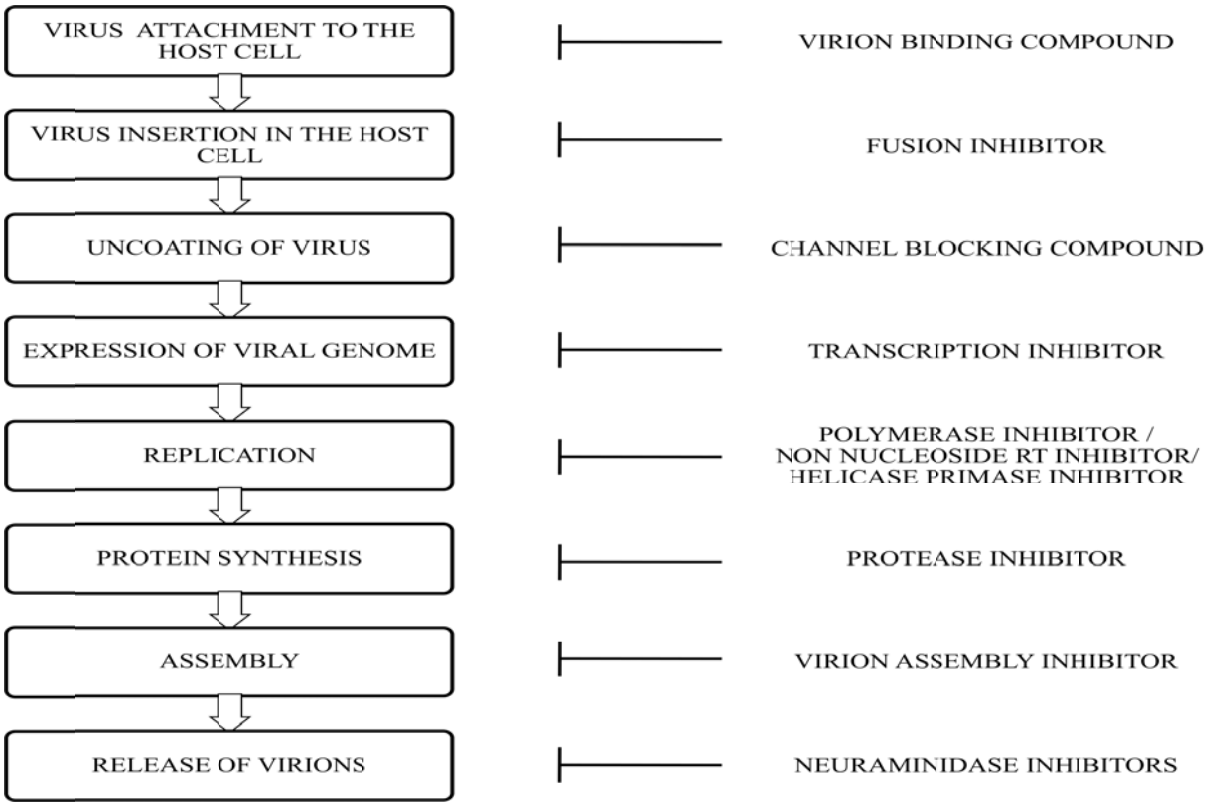

Fig. 1. Interaction of virus and drugs in the host cell (11)

3.5 Mechanism of action of Silver Nitrate

The mechanism for the antimicrobial action of silver ions is not properly understood. However the effect on bacteria can be observed by the structural and morphological changes. It is known that when DNA is in a relaxed state, replication of DNA can be effectively conducted. But when the DNA is in condensed form, it loses its replication ability. Therefore, when penetration of silver takes place inside the bacterial cell, the DNA molecule is reduced into condensed form and loses its replication ability leading to cell death. Also, some heavy metals react with proteins by getting attached with the thiol group and the proteins get inactivated (19).

3.6 Mechanism of action of silver nanoparticles

The silver nanoparticles show efficient antimicrobial property as compared to other salts due to their extremely large surface area. This enables them to achieve better contact with the microorganisms. Nanoparticles get adsorbed onto the cell membrane and also penetrate inside the bacteria. Silver nanoparticles interact with sulfur-containing proteins present in the cell membrane as well as with the phosphorus containing compounds like DNA. When entry of silver nanoparticles takes place inside a bacterial cell it forms a low molecular weight region in the center of the bacteria due to which the bacteria conglomerate. Thus shielding the DNA with the silver ions and inhibiting replication. The nanoparticles attack the respiratory chain and bring the cell division to an end resulting in cell death. The bactericidal activity of silver ions gets enhanced when they are released from nanoparticles in the bacterial cell (20-23).

3.7 Effect of size and shape of nanoparticles

The small size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells and therefore, it has higher efficiency than bigger particles (21). The nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles. Thus, it has been proved that the antibacterial activity of silver nanoparticles is size dependent (14). The antimicrobial efficacy of the nanoparticles is also dependent on the shape of the nanoparticles. This can be confirmed by studying the inhibition of bacterial growth by differently shaped nanoparticles (21). The content of silver in nanoparticles different shape is listed in Table 2. Therefore, the silver nanoparticles with different shapes have different effects on bacterial cell (24). A phenomenon called surface plasmon resonance plays a major role in the determination of optical density of metal nanoparticles. Particle size is proportional to the wavelength. The large surface area of nanoparticles will result in a higher intensity of interaction than larger particles having size in micrometers or more. Thus it is corroborated that the antibacterial effect of silver nanoparticles is size dependent.

Table 2: Shape and content of silver nanoparticles (24)

SHAPE OF NANOPARTICLE	TOTAL SILVER CONTENT
Triangular	1 µg
Spherical	12.5 µg
Rod Shaped	50-100 µg

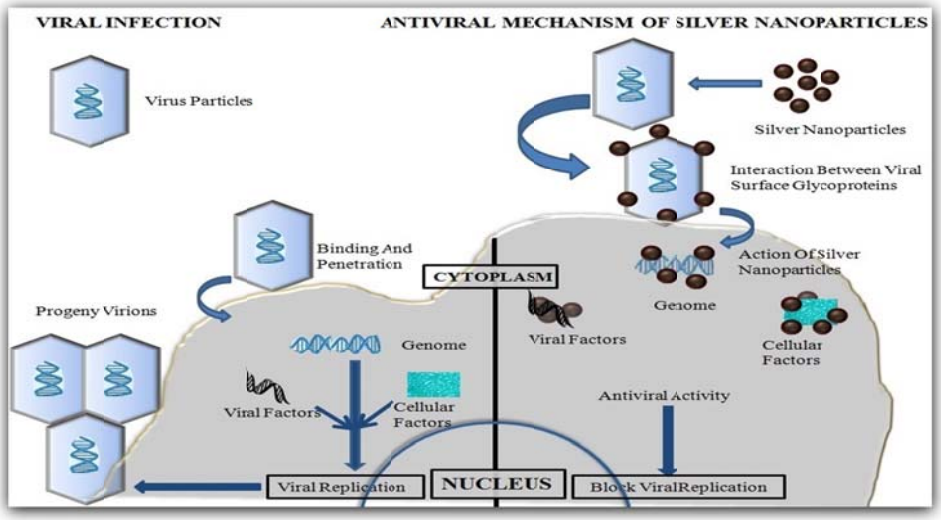

Fig. 2: Model of a virus infecting cell and antiviral mechanism of Silver nanoparticles (11)

3.8 Antiviral activity of Silver nanoparticles

Nanoparticles are used in study for their antimicrobial activity (antibacterial and antifungal activity). For the antiviral activity mainly two types of metallic nanoparticles are used; silver nanoparticles and gold nanoparticles. Silver nanoparticles are mainly used for the antiviral activity against viruses like HIV-1 (25-28), *hepatitis B virus* (29), *monkey pox virus* (8), *Tacaribe virus* (30), *influenza virus* (31), *herpes simplex virus* (32) *and respiratory syncytial virus* (33). For inhibition of viruses, nanoparticles of size ranging from 1-100 nm are mostly used (11). The interaction between silver and gold nanoparticles and the genome of the virus, a direct interaction with the viral surface proteins, is shown in figure 2. The size of the nanoparticles has a major role in the interaction; smaller the size more the interaction and more inhibition takes place. In addition nanoparticles come into the cell and apply their size dependent phenomenon which cause antiviral activity with their viral genome (DNA or RNA) (11). Smaller sized nanoparticles enter into the host cell and then enter in the viral genome where they block the cellular factors and/or the viral vectors which help in the viral replication. Alternatively, they may get attached to viral genome so that no polymerase action takes place and no further formation of progeny virions takes place.

Capping of silver and gold nanoparticles ensures a higher interaction rather than naked nanoparticles. Capping agents like Polysaccharides, polymers and Surfactants increase the efficacy of nanoparticles (34).

4. Silver nanoparticles against some deadly viruses

4.1 Human Immunodeficiency Virus Type 1

HIV belongs to *Retroviridae* family and causes AIDS (Acquired immunodeficiency Syndrome) which is a sexually transmitted disease. AIDS is caused by two *lentiviruses*, HIV-1 and HIV-2 (35). PVP coated nanoparticles have been used for the inhibition of HIV-1. These nanoparticles are synthesized by the polyol method using glycerol as reducing agent and as solvent. PVP is a linear polymer that stabilizes the nanoparticle surface by bonding with pyrrolidone ring. Silver nanoparticles have been conjugated with Bovine Serum Albumin (BSA), a polypeptide chain composed of 583 amino acid residues. Residues present in BSA like nitrogen, sulfur, oxygen bearing groups stabilize the silver nanoparticles. Strong interactions of silver nanoparticles are observed with the 35 thiol bearing cysteine residue (25). Sodium borohydrate is used for the direct bonding of BSA stabilized Silver nanoparticles with thiol bearing cystine residue. It also gives stearic protection.

The outer part of HIV-1 consists of a lipid membrane scattered with projecting glycoprotein knobs, formed by trimers which have two subunits. (1) gp 120 subunit exposed to the peripheral, and (2) gp 41 transmembrane glycoprotein which connects the gp 120 glycoprotein with the inner p17 matrix protein. The main function of gp 120 is binding with CD4 region on the host cell. Subunit gp 120 has 9 different disulfide bonds, out of which three are located in the neighborhood of CD4 binding domain (35). These bonds are the most striking sites for viral-nanoparticles interaction. Nanoparticles are located at particular location, having regular spatial correlation observed among three particles. The practical spatial arrangements show a relationship of the location of gp 120 glycoprotein knobs in the structural model for HIV-1 (25).

Regular spatial arrangements are determined by the HAADF (High Angle Annular Dark Field) scanning transmission electron microscopy. In the case of HIV-1 Virus, viral envelope mainly consists of a densely-packed lipid membrane. For the glycoprotein knobs, a localized region of lower density is observed due to the occurrence of membrane-spanning gp 41 glycoprotein. Therefore, glycoprotein areas appear darker than the remaining viral envelope. The centre to centre spacing is approximately 22 nm in glycoprotein knobs and spacing between silver nanoparticles is approximately 28 nm which is associated with the expected spacing between glycoprotein knobs. The experiential spatial arrangement of nanoparticles, the gap between nanoparticles and the uncovered sulfur-bearing remainder of the glycoprotein knobs are the striking sites for nanoparticles interaction suggesting that silver nanoparticles interact with the HIV-1 virus by means of better binding to the gp 120 glycoprotein knobs. The nanoparticles interact with the glycoprotein knob having size mainly 1-10 nm, sometimes 14 nm. Greater than this are not able to attach to the virus envelope (25).

4.2 Herpes Simplex Virus Type 1

HSV belongs to *Herpesviridae* family. It is a double stranded DNA virus having genome size 152Kbp. It is enclosed by isohedral capsids which are surrounded by a lipid bilayer envelope that encloses 11-12 virally encoded glycoproteins. HSV-1 is the most common infectious disease that occurs globally and infects humans. The result of HSV-1 includes a variety of clinical indications; a variety of asymptomatic infections to oral cold sores and severe encephalitis. The diameter of envelope is 170-200 nm and it contains an assortment of overhanging glycoprotein spikes which makes a full diameter of virions of 225 nm. Approximately 600-750 spikes having variable protective material concentration (32, 36, 37).

The entry of HSV takes place in the host cell when attachment of extracellular virions to the surface of the cell via glycoprotein C (gC) and Glycoprotein B (gB), which promote the binding of glycoprotein D (gD) to one of three alternative cellular receptors. In turn, membrane fusion machinery consist of gB, glycoprotein H (gH), and glycoprotein L (gL) that is triggered to arbitrate fusion with plasma or endocytotic membranes(37). For the duration of the attachment phase, gC and gB interact separately with cellular heparin sulfate (HS). This reversible interaction

probably creates various points of linkage and occurs in both naturally found and experimental viral strains. The affinity of the binding of gC to heparin sulfate is on the order of 10^{-8} M and is measured to be the key binding interaction during attachment. In the absence of gB and gC, comparatively low viral binding to the cell surface is seen which suggests the significant role of viral entry. Consequently, cells that are defective in HS demonstrate a remarkable reduction in susceptibility to infection. The interaction with cell surface HS has been found to be a common pathway for attachment by numerous other human and animal viruses (11, 32).

The mercaptoethane sulfonate capped silver nanoparticles and mercaptoethane sulfonate capped gold nanoparticles were used for the antiviral activity of the wild-type HSV-1 McIntyre strain. The viral inhibition indicates that silver and gold nanoparticles capped with MES block the attachment of HSV-1 to the host cell and thus the cell to cell infection is prevented. The spatially oriented functional groups are used due to the incapability of soluble MES and unchanged metal nanoparticles to control viral infectivity. Antiviral activity of Ag-MES and Au-MES nanoparticles proposes the opportunity of using substitute carrier core materials as well (11, 32, 36).

Table 3. Interaction of nanoparticles and possible mechanism (8, 11, 25-33, 36)

Virus	**Family**	**Metal Nanoparticles**	**Coating**	**Size**	**Mechanism of Action**
Human immunodeficiency virus type 1 (HIV-1)	*Retroviridae*	Silver Nanoparticles	PVP Coating	1-10 nm	Interaction with gp 120
					Bind with viral envelope glycoprotein
		Gold Nanoparticles	Mercaptobenzoic acid coating	2-20 nm	Inhibition of TAK-779 receptor
		Polymeric Nanoparticles	Core-corona Polymer	50 nm	Interaction with gp 120
Herpes Simplex Virus type 1 (HSV-1)	*Herpesviridae*	Silver Nanoparticles	MES coating	4 nm	Competition for the binding of the virus to the cell
		Gold nanoparticles			
Hepatitis B Virus (HBV)	*Hepadnaviridae*	Silver nanoparticles	–	10,50 nm	Interaction with double stranded DNA/ binding with viral particles
Monkey pox Virus	*Poxviridae*	Silver nanoparticles	Simple and Polysaccharide coating	10-80nm	Block of virus-host cell binding and penetration
Tacaribe virus (TCRV)	*Arenaviridae*	Silver Nanoparticles	Simple and polysaccharide coating	5-10 nm	Inactivation of virus particles before entry
Influenza virus	*Orthomyxoviridae*	Gold nanoparticles	Sialic acid functionalized	14 nm	Inhibition of virus binding to the plasma membrane
Respiratory syncytial virus	*Paramyxoviridae*	Silver Nanoparticles	PVP coating	69±3 nm	Interference with viral attachment

The viability of Vero cells against Ag-MES nanoparticles shows the 100% viability as MES coated silver nanoparticles did not affect the cell's mitochondrial activity at any concentration. Partial inhibition was shown when Ag-MES nanoparticles were given at the concentration of 200µg/ml and at the concentration of 400µg/ml complete inhibition was shown. At the concentration of 800µg/ml no further inhibition was shown (32).

4.3 Hepatitis B Virus

HBV belongs to *Hepadnaviridae* family having double stranded DNA genome which infects more than 400 million people in the world. The determination of HBV is linked with the growth of liver cirrhosis and hepatocellular carcinoma. When HBV enters into the hepatocyte, viral core of HBV is transferred to the nucleus where the HBV genome converts to covalently closed circular DNA which forms the mRNA and pre genomic RNA (pgRNA) for the transcription. pgRNA is further used for the reverse transcription and viral genome synthesis takes place. Six agents are used for the treatment of chronic HBV infection; (A) IFN-α2b, (B) IFN-α2a, (C) Lamivudine, (D) Adefovir, (E) Entecavir, and (F) Telbuvidine in which A & B are immunomodulatory agents named as conventional interferon. These aim to restore the host immune control. $CD8^+$ cytotoxic T lymphocytes and natural killer cells lyse the infected hepatocyte and are enhanced by IFNα. Synthesis of viral protein is also inhibited by the IFNα by modulating the action of antiviral cytokines. But IFNα creates severe side effects and low success rates. C, D, E & F are nucleos(t)ide analogues which act as specific inhibitors of viral polymerase reverse transcriptase (11, 29).

Silver nanoparticles of different diameters such as 10 nm and 50 nm interact with HBV dsDNA and viral particles are able to inhibit the viral replication and extracellular virions. The toxicity of silver nanoparticles against the HepAD38 cells was determined at the concentration of 5-50µM. The viability of cells decreases at 50µM for both 10nm and 50 nm silver nanoparticles. 800nm silver nanoparticles shows the severe toxicity at even 5µM due to the aggregation of silver nanoparticles. At the concentration of 5µM for both 10 nm and 50 nm silver nanoparticles the observed cell viability was 90 % (29).

For the 10nm silver nanoparticles the viral inhibition was 38% at 5µM and 80% at 50 µM. As well as 53% and 92% inhibition was shown at the concentration of 5µM and 50µM respectively for the 50nm silver nanoparticles. 10 nm silver nanoparticles showed good binding capacity to HBV virus which is shown by the transmission electron microscopy. Only 54% and 12% unbound virus were detected after 10 min and 60 min incubation respectively 10nm silver Nanoparticle was distributed in the cytoplasm and bind to the virus. Thus silver nanoparticles show high binding affinity for HBV dsDNA and extracellular virions (29).

4.4 Influenza virus

The influenza virus is a highly pathogenic *orthomyxovirus* that causes annual pandemic in the human Population worldwide. Recently, gold nanoparticles were used to inhibit the influenza virus. It contains a helical capsid having genome of eight RNA segments. It is covered by a lipid envelope having two virally-encoded glycoproteins, haemeagglutinin (HA) and neuraminidase (NA) that forms sharp protrusion on the surface. It binds to the plasma membrane of the host cell through contact between HA and Sialic acid (SA) residues which are present on glycoprotein and lipids. The mechanism of receptor-mediated endocytosis brings the enclosed virus particle within the cytoplasm. Inside the late endosome, atmospheric acidification activates a conformational change of HA, which mediates the protein fusion of the endosomal membrane with the viral covering ending with the discharge of the nucleoproteins and genome fragments into the cytoplasm (10, 11, 31).

Gold nanoparticles of different sizes, 2nm and 14 nm were used in which only 14 nm gold nanoparticles inactivate haemagglutination at attentiveness in the nano molar range. They demonstrate that the activity depends on the particle size of the interacting ligand/receptor molecules. Therefore, it has been proven that Sialic-acid-functionalized gold nanoparticles are capable to successfully reduce viral infection (31).

4.5 Monkey pox virus

MPV, an *orthopox virus*; is a big threat to human life. However, more investigation needs to be done for bringing out the exact mechanism for the inhibition of virus with the help of Silver nanoparticles. Poxvirus enters into the host cell by the endocytosis or by the direct fusion with the plasma membrane, followed by a regulated sequence of events leading to the viral replication. The sizes of silver nanoparticles for the MPV inhibition are 10 nm, 25 nm and 80 nm. The internalization of silver nanoparticles suggests that a possibility is there for the disruption of intracellular pathway that attenuates the viral replication (8).

4.6 Tacaribe virus

TCRV belongs to the family *Arenaviridae*. It contains 18 different species which are divided into two antigenic groups' namely old world and new world. New world is also known as *Tacaribe* complex which contains numerous viruses such as *Junín, machupo, guanarito*, and *sabia*. TCRV is closely related to the *Junín* and *guanarito* viruses. *Arenaviruses* doesn't have any vaccine yet (30). Two types of silver nanoparticles are used for the inhibition of TCRV which are (A) uncoated silver nanoparticles (Ag-NP), and (B) polysaccharide coated silver nanoparticles (PS-Ag) (11, 30).

Silver nanoparticles bind to glycoproteins present in the viral membrane. Glycoproteins of TCRV are cysteine rich residues (38) and silver nanoparticles can easily bind to the thiol groups, present in cysteine residues (39). This interaction causes three phenomenon: (A) It can interfere in the receptor binding and avoid the internalization of the viral particle, (B) It internalizes the silver nanoparticles which inhibit the viral replication of TCRV L protein, (C) silver nanoparticles get attached to the viral glycoprotein and prevent the viral coating in the endosome. Cells treated with the silver nanoparticles has no effect on viral replication, so Ag-NPs inactivates the virus before the entry in the cell (30).

Silver nanoparticles show the 25% decline in cell viability when the Vero cells were exposed up to 50µg/ml. There was no any reduction shown in 50µg/ml but when the cells treated with 75µg/ml and 100µg/ml; 60% reduction in cell viability is shown and cell died by day 2. When TCRV was treated with uncoated silver nanoparticles having size 10 nm, a significant reduction of virus was seen. Polysaccharide coated silver nanoparticles show less reduction but also have low toxicity to the cell as compared to the uncoated silver nanoparticles. Polysaccharide coating protects the cell from the toxic effects of uncoated silver nanoparticles but interference is seen against TCRV. When TCRV is treated with 10µg/ml then 50% reduction was shown in progeny virus. At the concentration of 25µg/ml no detectable progeny were seen.

Real Time PCR, Confocal Microscopy, and Transmission Electron Microscopy (TEM) shows the activity of silver nanoparticles against the *Tacaribe* virus. The Confocal microscopy and transmission electron microscopy show the internalization of TCRV into the Vero cells; 10nm silver nanoparticles were able to enter into the cells and also in the same endosome while the 25nm silver nanoparticles enter into the individual endosome.

Real Time PCR shows the quantitative results which shows that silver nanoparticles inhibit the virus at the concentration of 25µg/ml and 50µg/ml all silver nanoparticles shows the significant reduction in the amount of S segment expression (30).

4.7 Respiratory Syncytial Virus

RSV belongs to the family *Paramyxoviridae.* It infects the lungs' epithelium and the respiratory tract which causes serious respiratory diseases in children. There is no vaccine available for the treatment of RSV. Its genome is a single RNA molecule of negative-sense RNA, which codes two surface glycoproteins (protein G and protein F) exposed on the viral envelope. G protein is responsible for receptor binding protein, and F protein serves for the fusion between the cell membrane and the viral envelope. F protein is expressed on the cell surface and fuses adjacent cells and makes syncytia formation, a well known cytopathic effect (40).

Silver nanoparticles are being utilized to study the inhibition of RSV. The capping agents are (1) poly(N-vinyl-2-pyrrolidone) (PVP); (2) bovine serum albumin (BSA); and (3) a recombinant F protein from RSV (RF 412) for the silver nanoparticles (33). The TEM analysis indicates that the (1) BSA-conjugated silver nanoparticles interact with RSV without exact

organization or spatial arrangement, (2) RF 412 coated silver nanoparticles are suspended liberally without regular attachment (3) PVP-coated silver nanoparticles bind to the viral surface that has regular spatial arrangement and attached G proteins. They interact with the G proteins and get dispersed on the RSV virions. When cells interact with BSA, PVP and RF-412 coated silver nanoparticles, inhibition is categorized by immunofluorescence microscopy, no inhibition is shown by BSA and RF-412 coated nanoparticles, whereas PVP coated silver nanoparticles inhibit 44% infection (33).

5. Other useful applications

It is seen that Silver nanoparticles have the antiviral and antimicrobial activities against several pathogens. Their utility is incorporated in materials and biomedical applications (41). Nanoparticles are used as additives in health care such as bandages and catheters for healing of wounds and burns in less time. Ag/Na carboxymethyl cotton burn dressing is used for the applications in surgical dressings. They are also used in common products such as water purification systems, domestic products, cosmetic products and emulsions to prevent harmful micro flora (42).

6. Conclusion

In this era, when drug and vaccine development for the removal of various viral diseases is riding high, some viral strains have emerged that are resistant against the drugs and vaccines, like HIV. So it is important to introduce the multidisciplinary approaches with the classical epidemiology, along with the clinical phases to introduce a new drug or vaccine which proves highly beneficial against the resistant strain. Nanotechnology is the one that gives the opportunity to re-discover biological properties of ancient antimicrobial and antiviral compounds. Nanoparticles, mainly silver have antiviral activities against the many viruses of today that are playing havoc with lives worldwide. Extensive research and clinical trials need to be carried out so as to accentuate the efficacy of this medical marvel towards betterment of the health of the global population.

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